

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Kwon, Byoung
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Title: Human H4-1BB Protein

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AFFIDAVIT FOR PRIORITY SHOWING UNDER 37 CFR 41.202(d)(1)

Commissioner of Patents
P.O. Box 1450
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Sir:

I, Kack-Kyun Kim declare and state as follows:

1. I am currently a Professor at the Department of Oral Microbiology/Immunology, Seoul National University School of Dentistry, Seoul, Republic of Korea. Prior to May 7, 1993, I had performed experiments at the laboratory of Dr. Byoung Kwon as a visiting scientist, then at Indiana University School of Medicine, Indianapolis, Indiana.
2. I have no financial interest in the outcome of the patent application referenced.
3. I received a D.D.S. in 1978, a M.S. in Dental Pharmacology and Therapeutics in 1980, and a Ph.D. in Microbiology in 1983 from Seoul National University, Seoul, Korea. From 1981-1983, I was an Assistant in the College of Dentistry, Seoul National University, Seoul, Korea. I served Republic of Korea Army as a dental officer from 1983-1986. From 1987-1991, I was a Full-time Instructor in the Department of Oral Microbiology, College of Dentistry, Seoul National University. From 1991-1997, I was an Assistant Professor in that same Department. From 1997-2006, I was an Associate Professor in that same Department. I am currently a Professor in the Department of Oral Microbiology/Immunology. I have authored or co-authored 32 papers internationally and 25 papers domestically, primarily in the areas of oral microbiology and immunology.
4. I provide this affidavit describing my work at Dr. Kwon's laboratory, as evidence in support of priority for the patentability of pending claims of the application referenced above. I

was involved in a variety of projects in Dr. Kwon's laboratory during my stays there as a visiting scientist and some of the projects I was involved, related to the cloning of human 4-1BB fragment in expression vectors. My contributions for cloning human 4-1BB fragments in expression vectors and their characterization were recognized in the published journal article Zhou et al., (1995), Characterization of human homologue of 4-1BB and its ligand, *Immunol Lett.*; 45(1-2):67-73, (EXHIBIT 13, KWON000105-111), in which I am one of the listed authors.

5. I understand that the priority filing date of May 7, 1993 for the cited reference, the Goodwin et al., (U.S. Patent No. 7,211,259), is the critical date to demonstrate priority for the application referenced-above.

6. Prior to May 7, 1993, under the guidance and request of Dr. Byoung Kwon, I performed the cloning of human 4-1BB into a human placental alkaline phosphatase tag (APtag-1) expression vector. (EXHIBIT 16, KWON000125-128). EXHIBIT 16 is a copy of pages from one of the laboratory notebooks that I maintained during my stay at Dr. Kwon's laboratory. The alkaline phosphatase-4-1BB fusion protein was useful in determining the relative amount of the 4-1BB protein present in the various cell types. (EXHIBIT 13, KWON000110). The APTag vector and the human 4-1BB PCR products were digested with restriction enzymes BglII and HindIII for directional cloning, based on the design of primers by Dr. Kwon. (EXHIBIT 15, KWON000123-124). As shown in the gel picture of EXHIBIT 16 (KWON000128), APTag vector and human 4-1BB fragment digested with BglII and HindIII show distinct bands for the lanes marked as APtag/HindIII-BglII and 4-1BB (PCR)/ Hind III-BglII. The restricted vector and the PCR product (HindIII-BglII 4-1BB fragment) were gel purified and a ligation reaction was set-up with a ligase. (EXHIBIT 16, KWON000125-127). The human 4-1BB fragment that contained the extracellular domain was cloned in front of the coding sequence of the alkaline phosphatase in the mammalian expression vector. (EXHIBIT 13, KWON000106). The resulting construct was designated 4-1BB-AP as mentioned in EXHIBIT 13 (KWON000106) and EXHIBIT 12 (KWON000088). Subsequently, I understand that the fusion protein containing construct, the 4-1BB-AP plasmid, was linearized with a restriction enzyme ClaI and was co-transfected with a linearized selectable marker plasmid, pSV7neo, by calcium phosphate co-precipitation method, as mentioned in EXHIBIT 13 (KWON000106).

7. Prior to May 7, 1993, I was also involved in cloning an EcoRI digested human 4-1BB fragment into an expression vector pXM that was also digested with EcoRI and treated with calf-intestinal phosphatase (CIP). (EXHIBIT 18, KWON000158-160, and KWON000166). EXHIBIT 18 is a copy of one of the laboratory notebooks that I maintained during my stay at Dr. Kwon's lab. Initially, as mentioned in EXHIBIT 18 (KWON000158-160), the ligation reaction did not work. However, I repeated the ligation of EcoRI-digested human 4-1BB fragment with CIP-treated EcoRI digested pXM vector (EXHIBIT 18, KWON000166). Nevertheless, the experiments demonstrate that a human 4-1BB fragment was available for various cloning experiments that I performed for subsequent expression studies.

8. In addition to generating fusion proteins of human 4-1BB, prior to May 7, 1993, I was involved in experiments designed to investigate homology analysis of 4-1BB sequence in other species, including mouse and human. (EXHIBIT 18, KWON000130). Prior to May 7, 1993, I used a human 4-1BB fragment as a probe to hybridize blots containing DNA isolated from a variety of species including mouse, gibbon, and human DNA that were restricted with EcoRI (EXHIBIT 18, KWON000130-132). In addition, I performed amplifications of total and polyA RNA using human 4-1BB primers. MLA polyA⁺ refers to Gibbon T-cell RNA and Jurkat and Molt4 refer to T-cell lines of human origin. Prior to May 7, 1993, I labeled a 1.2 Kb fragment of human 4-1BB with radioactive ATP and CTP by a nick-translation method. (EXHIBIT 18, KWON000142). The labeled probe was then used to hybridize Southern blots containing DNA from mouse, Gibbon and human samples. I performed the hybridizations at high stringency at 65°C over night. After a high-stringency washing, I exposed the blot for 4 days. Prior to May 7, 1993 I performed a dot blot analysis of PCR products from the amplification of MLA total/polyA⁺ RNA by probing with labeled human 4-1BB fragment. (EXHIBIT 18, KWON000145-149). These experiments demonstrate that a human 4-1BB fragment was available for homology studies and was used in the labeling experiments for dot blot analysis.

9. Therefore, in summary, I state that, prior to May 7, 1993, I used a human 4-1BB nucleotide fragment for directional cloning of fusion proteins of human 4-1BB into expression vectors and for homology studies based on hybridization of labeled human 4-1BB probes.

10. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: September 1, 2008

By: Kuckkyun Kim

Kuck-Kyun Kim